

AD-772 965

INSECT CHEMICAL DETECTION

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Army Land Warfare Laboratory
Aberdeen Proving Ground, Maryland

November 1973

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UNCLASSIFIED
Security Classification

AD 772965

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Biomedical Laboratory Edgewood Arsenal, Md		2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	
		2b. GROUP	
3. REPORT TITLE INSECT CHEMICAL DETECTION			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Final Report			
5. AUTHOR(S) (First name, middle initial, last name) Kenneth Frumkin, ILT Kenneth Zych, CPT			
6. REPORT DATE November 1973		7a. TOTAL NO. OF PAGES 10	7b. NO. OF PAGES 5
8a. CONTRACT OR GRANT NO.		9a. ORIGINATOR'S REPORT NUMBER(S) na	
b. PROJECT NO.			
c.		9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) na	
d.			
10. DISTRIBUTION STATEMENT Approved for public release; distribution unlimited.			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY USA Land Warfare Laboratory Aberdeen Proving Ground, MD 21005	
13. ABSTRACT <p>Thorpe (1939) found that fruitflies (<u>Drosophila melanogaster</u>) reared on a medium containing peppermint oil would show a preference for this substance as adults, whereas normally-reared flies avoid peppermint oil. The practical applicability of this phenomenon for narcotic detection was tested by rearing <u>Drosophila</u> on a medium containing either marijuana or heroin. When released in a 6' x 1' x 1' screened chamber designed to approximate an open room, flies reared on narcotics showed no tendency to approach them.</p>			

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NOV 65 1473 OBSOLETE FOR ARMY USE.

UNCLASSIFIED
Security Classification

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TECHNICAL REPORT NO. LWL-CR-08B73

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Final Report

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ABSTRACT

Thorpe (1939) found that fruitflies (Drosophila melanogaster) reared on a medium containing peppermint oil would show a preference for this substance as adults, whereas normally-reared flies avoid peppermint oil. The practical applicability of this phenomenon for narcotic detection was tested by rearing Drosophila on a medium containing either marijuana or heroin. When released in a 6' x 1' x 1' screened chamber designed to approximate an open room, flies reared on narcotics showed no tendency to approach them.

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INTRODUCTION

The unique sensitivity of insects as chemical detectors has been studied extensively (see Dethier, 1947). Most research has looked at the responses of various insects to substances for which they have natural preferences or aversions. However, at least one method has been demonstrated for altering the natural responses of fruit flies (Drosophila melanogaster) to certain chemicals. By raising Drosophila larvae on a medium containing peppermint oil, Thorpe (1939) was able to reverse the normal aversion to this substance shown by adult flies. That is, Drosophila reared with peppermint oil in the growth medium showed a slight preference for the odor of peppermint as adults, whereas normally reared flies avoid this odor. While there are some very important theoretical questions regarding the nature of this change in response, there is no doubt that the Thorpe (1939) experiment can be successfully replicated (see Hershberger and Smith, 1967; Manning, 1967; and Thorpe, 1956).

The present research represents an attempt to apply this procedure to ascertain whether fruit flies reared on a medium containing a narcotic drug can be used to detect the presence of that drug in a more natural setting.

In order to test the generality and applicability of Thorpe's (1939) findings, Drosophila larvae were fed on a medium containing either marijuana or heroin. The adult flies were then released, several hundred at a time, into the middle of a 6' x 1' x 1' screened chamber. A quantity of the appropriate drug (the "target material") was located at one end. After several hours, the distribution of flies in the apparatus was determined. It was felt that this technique would more closely approximate a totally open environment while still allowing for quantification and control. In contrast, the olfactometers used in the peppermint-oil experiments allowed for studying the responses of only one fly at a time in an area of a few cubic centimeters.

CONCLUSION

Contrary to what might have been expected from Thorpe's (1939) experiments, flies reared on a medium containing either marijuana or heroin showed no tendency to approach them.

METHOD AND PROCEDURE

Subjects. Randomly bred Drosophila melanogaster were used without regard to sex. The flies were reared in cylindrical plastic vials (4" x 1.25" in diameter) closed with plastic foam stoppers. Each vial contained 5 gm. of commercial Drosophila medium.¹ Flies were housed

¹Carolina Biological Supply Co., No. 67-5002.

and tested in rooms with controlled temperature and humidity (average temperature and humidity on test days were 25°C and 60%). Most of the flies were tested within 72 hours of emergence as adults.

Apparatus. The apparatus was a 6' x 1' x 1' (interior dimensions) rectangular wooden box supported on four legs (see Figure 1). The top of the box was 4 ft. above the floor of the room. The front side was made of fine wire screening. Six evenly spaced 2-inch diameter holes in the back of the apparatus were also covered with screening. The top was hinged, and the floor of the inside was painted white. There was a 1.25-inch hole approximately 1 inch to one side of the center of the floor.² Grooves were cut in the back, top and bottom of the apparatus to admit five evenly-spaced sliding Masonite panels (1' x 1' x .12") that divided the interior of the apparatus into six one-cubic-foot compartments. The apparatus was placed in the center of a small room (10' x 10'), lighted with two 4-tube fluorescent fixtures. Since room light entered the box through the screened front, the apparatus was oriented so that the light fixtures were equidistant from the ends.

Procedure. Regardless of the target material employed, the general procedure was the same. Immediately before testing, the flies were transferred from a number of vials into an empty opaque vial. The apparatus was closed, the masonite panels withdrawn, and the vial inserted into the hole in the floor of the apparatus. Since most of the flies were positively phototropic and negatively geotaxic, darkening the "start" vial and placing it underneath the apparatus facilitated their entry into the chamber. The apparatus and experimental room were then left undisturbed for a time ranging from 1 to 5.5 hours. (Most tests were 2 to 2.5 hours). At the end of the test period the dividers were quickly inserted. The flies were then killed with carbon dioxide directed through the wire screening and the number of flies in each of the six compartments were counted. This was usually done by two independent observers whose counts were then averaged.

TESTS AND RESULTS

Experiment 1: Blank Trials

Method. Four separate tests were run with no target material (drug) in the apparatus. This was done in order to find out if the flies would be distributed normally about the apparatus when released in the center. Test duration was also varied. A total of 1077 "normal" flies (those reared on standard medium) were tested as described above.

Results and discussion. Table 1 presents the data from the four tests in order of increasing length. The numbers represent the percentage of the total number of flies released that was found in each of the sections of the apparatus. It is clear from the distributions obtained that there

²This represents an error in apparatus construction. The hole should have been centered.

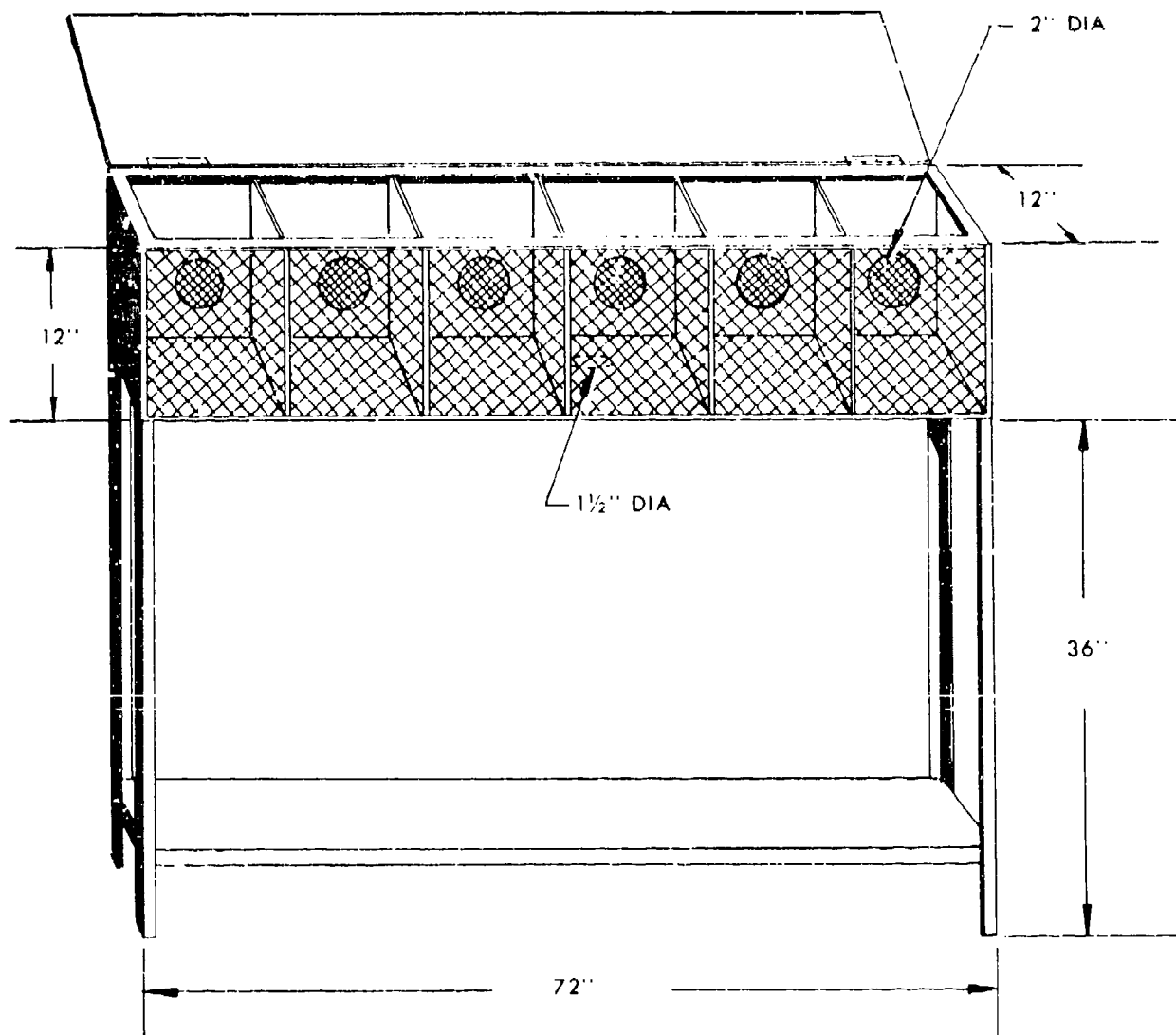


FIGURE 1. Apparatus.

Table 1. Percentage of Flies Found in Each Apparatus Section on Tests with No Target Material

Test	Total Time (min)	Number of flies	Apparatus Section					
			1*	2	3	4	5	6
1	75	29	3.4	6.9	31.0	34.5	20.7	3.4
2	100	422	6.2	14.0	24.9	34.8	11.1	9.0
3	150	509	7.9	11.2	24.6	27.3	19.4	9.6
4	165	117	7.7	12.0	23.1	26.5	14.5	16.2
Mean percentage per section:			7.1	12.3	24.7	30.4	15.7	9.9

*Location of target material on subsequent tests.

was a consistent tendency for more flies to go to one side of the apparatus than to the other. Considering the flies still in the middle two sections of the apparatus at the end of test as having "no preference" a significant majority of the remaining flies went to boxes 5 and 6 ($\chi^2 = 10.17$, $df = 1$, $p < .01$). It is likely that this bias reflects the placement of the starting hole slightly toward this side. Because of the trend, it was decided to place the target materials used in the subsequent experiments in Box 1. Any tendency for subjects to approach the target could not, then, be attributed to this bias in the apparatus.

Experiment 2: Marijuana

Method. Sixteen vials of flies were reared on the standard medium for this experiment. Eight of the vials also contained 0.294 gm. of marijuana (ground leaves and seeds). The marijuana powder was assayed at 1.7% tetrahydrocannabinol (THC, the active ingredient in cannabis), making a concentration of 5 mg. THC/5 gm. of medium (0.1% THC).

Testing was identical for experimental and control groups. Five grams of the marijuana powder were placed in a loosely closed paper envelope (9-1/2" x 5") placed in the center of Box 1. (The experimenters could easily detect the odor of the target material through the envelope.) An empty envelope was left at the other end of the apparatus. Flies from only one group were released each time. Two control and three experimental tests were conducted.

Results and Discussion. The results of the second experiment are presented in Table 2. The percentage of flies found in each of the six boxes is given for each trial. Control trials are presented at the top of the Table, and experimental trials are on the bottom. The control flies in this experiment did not display any side preferences at all. The proportion going to the side containing marijuana (sections 1 and 2 of the apparatus) was not significantly different from the distribution in the other end of the apparatus (24.5 vs. 24.6%, $\chi^2 = 0.002$, $df = 1$, $p > .10$).

The flies in the experimental group, on the other hand, were more likely to go to the empty side of the apparatus ($\chi^2 = 12.4$, $df = 1$, $p < .001$).

It is not clear why flies in the control group in this experiment behaved differently from those in Experiment 1. Perhaps the factors producing the apparatus bias found in the first experiment had changed. Flies not previously exposed to marijuana may even have had enough of an attraction toward it to counteract the bias in the apparatus. However, the important finding is unambiguous: Contrary to what might have been expected from Thorpe's (1939) experiments, flies in the experimental group did not approach the marijuana.

Table 2. Percentage of Flies Found in Each Apparatus Section:
Control vs. Flies Reared on Marijuana

Test	Total Time	Number of flies	Apparatus Section					
			1*	2	3	4	5	6
(min)								
<u>CONTROL</u>								
1	120	107	7.5	13.1	25.2	40.2	9.3	4.7
2	150	900	10.1	15.0	26.8	22.3	16.2	9.5
			—	—	—	—	—	—
Mean percentage per section:			9.8	14.8	26.6	24.2	15.5	9.0
<u>EXPERIMENTAL</u>								
1	60	223	0.0	1.8	26.9	64.6	5.8	0.9
2	135	379	7.4	8.2	29.3	31.9	14.5	8.7
3	150	332	5.7	12.7	21.7	34.9	15.1	9.9
			—	—	—	—	—	—
Mean percentage per section:			5.0	8.2	26.0	40.7	12.6	7.3

*Location of target material.

Experiment 3: Heroin

Method. Thirty-seven vials of flies (25 experimental, 12 control) were reared and tested for this experiment. Experimental vials contained 50 mg. of heroin powder (assayed as 98% pure). The target was 2.0 gm of heroin powder in a plastic bottle having a perforated lid. The bottle was loosely enclosed in a paper envelope in Section 1. An empty envelope was again placed in Section 6.

Results and Discussion. Table 3 presents the findings from three control and five experimental tests. The results from the control group are similar to those for the blank trials (Table 1): more flies went to the non-target side on every trial (23.3 vs. 19.3%, $\chi^2 = 5.42$, $df = 1$, $p < .02$). On the other hand, flies in the experimental group did not show any significant preference (Sections 1 and 2 = 20.4%, Sections 5 and 6 = 19.7%, $\chi^2 = 0.25$, $df = 1$, $p > .10$).

For heroin, then, as well as for marijuana, there was no indication that flies reared on a narcotic drug would seek that drug in an open environment.

DISCUSSION

The present experiments provide no evidence that Drosophila melanogaster will seek out either marijuana or heroin when the drug had been present in their larval growth medium. From a purely scientific point of view, these negative findings do not rule out the possibility that tests more similar to Thorpe's would lead to different results. However, the question of practical applications of this procedure to narcotic detection seems to have been answered unequivocally. If no preference could be demonstrated within the apparatus used, it is unlikely that tests in a more open environment would be productive.

Several factors decrease the likelihood of successful narcotic detection using this technique. The most important limiting factor involves the question of the very nature of the phenomenon observed by Thorpe. There are two possible interpretations of his results: The apparent attraction for peppermint shown by fruitflies reared on a medium containing that substance may be due to conditioning. In this case, the flies' behavior represents a learned attraction for the odor that is based on its association with feeding (Hershberger and Smith, 1967). On the other hand, Thorpe's findings may not represent a learned preference at all, but rather habituation³ to the normally aversive properties of the peppermint oil. If the first possibility (learning) was the correct interpretation, then one might reasonably expect flies to seek out substances on which they were reared, because of their acquired reward value. However, if the flies' behavior in the Thorpe (1939) paradigm is based solely on habituation, then flies exposed to narcotics as larvae can merely be expected to be more neutral towards them than

³Habituation is defined as the decrease in responsiveness to a stimulus that occurs as a result of repeated presentations.

Table 3. Percentage of Flies Found in Each Apparatus Section:
Control vs. Flies Reared on Heroin

Test	Total Time (min)	Number of Flies	1*	2	3	4	5	6
<u>CONTROL</u>								
1	120	283	4.2	5.7	25.4	48.8	7.1	8.8
2	150	593	6.9	14.8	28.5	24.8	16.4	8.6
3	210	579	9.7	11.7	24.7	29.2	13.8	11.4
Mean percentage per section:			7.5	11.8	26.2	31.2	13.5	9.8
<u>EXPERIMENTAL</u>								
1	120	505	4.2	12.5	29.9	37.4	13.5	2.6
2	150	337	8.6	12.1	25.8	32.6	14.2	6.5
3	150	374	4.8	13.6	29.7	34.0	13.4	4.5
4	165	607	11.4	15.2	21.1	29.7	15.2	7.6
5	330	166	4.2	9.0	24.7	40.4	12.7	9.0
Mean percentage per section:			7.2	13.2	26.0	33.8	14.0	5.7

*Location of target material.

unexposed flies. In an open environment they would have no approach tendency.

The best evidence favors the habituation interpretation. Thorpe himself (1959) explained his earlier findings in this way. In addition, strong evidence comes from Manning (1967), who first replicated Thorpe's study and then tested the flies that had chosen the peppermint-oil arm for a second trial. On that trial, the choices of the flies that presumably "preferred" peppermint were completely random. These findings imply strongly that there was no learned preference, but rather an absence of the aversion to peppermint that was shown by the control flies (habituation).

Another serious problem is the relative insolubility of both marijuana and heroin in the growth medium. While no information exists on the effects of this variable on the Thorpe (1939) phenomenon, it is not unlikely that solubility may play a significant role.

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